

In The Specification

Amend the first paragraph at page 2, line 3, as follows:

This application is a continuation of co-pending application Serial No. 09/440,772, filed November 16, 1999, which claims priority to U.S. provisional patent application Serial Number 60/108,563, filed on November 16, 1998, and U.S. provisional patent application Serial Number 60/115,175, filed on January 8, 1999, both of which are herein incorporated by reference.

Please substitute the paragraph extending from page 5 line 22 through page 6 line 3 for:

In another embodiment, the method of the present invention provides a target cell that presents at least an HIV polypeptide, which includes the HIV envelope (env) polypeptide or the gag polypeptide, in addition to HIV polypeptide fragments thereof. In a preferred embodiment, the polypeptide is gp160, or fragments thereof. In further embodiments, the invention predicts long-term non-progression of AIDS by using a target cell that presents a synthetic peptide whose amino acid sequence is derived from an HIV gene product such as a synthetic peptide, which can be from 11 to 25 residues in length. In additional embodiments, the peptide sequences include YL(R/K)DQQLLGIWGC (SEQ ID NO:33 or SEQ ID NO:34), FLGFLGAAGSTMGAASLTQVQARQ (SEQ ID NO:20), or VYYGVPVWKEA (SEQ ID NO:1).

Please substitute the paragraph extending from page 6 line 10 through page 7 line 6 for:

The present invention next provides a method of preventing an HIV-infected subject from developing AIDS by determining whether the patient expresses HLA-Cw7 and demonstrates an HLA-Cw7-restricted, HIV-specific CTL response; if such a response is exhibited, the patient is administered a composition that contains an HIV polypeptide that is also an HIV CTL epitope. Alternatively, the methods of the invention can be practiced by determining whether the HIV-infected subject has an HLA-Cw7 haplotype. The method is understood to encompass patients who are infected with HIV-1. A composition of the claimed invention includes HIV polypeptides such as the env polypeptide, the gag polypeptide, and fragments of either.

Furthermore, the HIV polypeptide of the claimed invention further is understood to include a synthetic peptide whose sequence is derived from HIV gene products. Such a synthetic peptide can be from 11 to 25 residues in length and could include sequences such as YL(R/K)DQQLLGIWGC (SEQ ID NO:33 or SEQ ID NO:34), FLGFLGAAGSTMGAASLTQTVQARQ (SEQ ID NO:20), or VYYGVPVWKEA (SEQ ID NO:1). The method also could include administering a plurality of HIV polypeptides. This plurality of HIV peptides could include 2 or more different peptides containing the sequences YL(R/K)DQQLLGIWGC (SEQ ID NO:33 or SEQ ID NO:34), FLGFLGAAGSTMGAASLTQTVQARQ (SEQ ID NO:20), or VYYGVPVWKEA (SEQ ID NO:1). Alternatively, the method could include administering one or more synthetic peptides from 11 to 25 residues in length that include sequences such as YL(R/K)DQQLLGIWGC (SEQ ID NO:33 or SEQ ID NO:34), FLGFLGAAGSTMGAASLTQTVQARQ (SEQ ID NO:20), VYYGVPVWKEA (SEQ ID NO:1), LWDQSLKPCVKLT (SEQ ID NO:4), SVITQACSKVSFE (SEQ ID NO:8), or GTGPCTNVSTVQC (SEQ ID NO:16). A plurality of peptides that comprises, two, three, four, five or all six of these sequences is included within the methods of the present invention. It is further contemplated that the sequences may also be included in peptides that have additional residues flanking one or more ends of the sequences. For example, the peptide FLGFLGAAGSTMGAASLTQTVQARC (SEQ ID NO:35) falls within the scope of the present invention.

Please substitute the paragraph extending from page 7 line 26 through page 8 line 17 for:

The present invention also includes a method of preventing HIV infections in an uninfected subject by first determining whether the subject has or expresses an HLA-Cw7-haplotype and, if the subject does, then administering to the subject a composition containing an HIV polypeptide that also is a CTL epitope, optionally also providing a T helper epitope. This preventative method contemplates prevention of infection by HIV-1. If the subject who is uninfected can express an HLA-Cw7 haplotype, the invention is understood to include compositions of an HIV polypeptide encompassing HIV envelope polypeptide or gag polypeptide, or fragments thereof. A synthetic peptide whose sequence is derived from an HIV polypeptide also can be used. This HIV-derived synthetic peptide can be from 11 to 25 residues

in length and include the sequence YL(R/K)DQQLLGIWGC (SEQ ID NO:33 or SEQ ID NO:34), FLGFLGAAGSTMGAASLTQTVQARQ (SEQ ID NO:20), or VYYGVPVWKEA (SEQ ID NO:1). As previously mentioned, the methods of the present invention also include peptides comprising one or more of the following sequences: LWDQSLKPCVKLT (SEQ ID NO:4), SVITQACSKVSFE (SEQ ID NO:8), or GTGPCTNVSTVQC (SEQ ID NO:16). Any combination of one, two, three, four, five, or six of these peptide sequences may be used with the methods of the present invention. Furthermore, the HIV polypeptide can be coupled to a carrier such as KLH or BSA; and, it could also be administered with an adjuvant, where the adjuvant is a lipid, a toxin, cytokine synthetic [oligonucleotide] oligonucleotide or bacterial DNA. In addition to administering to the uninfected subject an HIV polypeptide, the subject also can be treated with AZT or HAART.

Please substitute the paragraph extending from page 8 line 18 through line 27 for:

As previously mentioned, the HIV peptides used in the methods of the present invention may be provided to a cell as an expression construct that comprises a polynucleotide encoding one or more HIV peptides. In some aspects of the present invention, different mini-gene constructs may be administered such that more than one type of peptide sequence is provided to a cell. In other aspects of the present invention, an expression construct may contain sequences that enable it to express more than one peptide sequence; for example, the expression construct may contain sequences that allow it to express both FLGFLGAAGSTMGAASLTQTVQARQ (SEQ ID NO:20) and VYYGVPVWKEA (SEQ ID NO:1). The expression construct may thus be able to express one, two, three, four, five, six or more peptide sequences.

Please replace the table on pages 44 and 45 with the following table:

TABLE 2
Peptide Sequences

Peptide 104	V Y Y G V P V W K E A	<u>SEQ ID NO:1</u>
Consensus-A	V Y Y G V P V W K D A	<u>SEQ ID NO:2</u>
Consensus-B	V Y Y G V P V W K E A	<u>SEQ ID NO:1</u>
Consensus-C	V Y Y G V P V W K E A	<u>SEQ ID NO:1</u>
Consensus-D	V Y Y G V P V W K E A	<u>SEQ ID NO:1</u>
Consensus-E	V Y Y G V P V W R D A	<u>SEQ ID NO:2</u>
Consensus-F	V Y Y G Y P V W K E A	<u>SEQ ID NO:1</u>
Consensus-G	V Y Y G Y P V W E D A	<u>SEQ ID NO:2</u>
Consensus-O	V Y S G V P V W E D A	<u>SEQ ID NO:3</u>
Consensus-U	V Y Y G V P V W K D A	<u>SEQ ID NO:2</u>
Peptide 111	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-A	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-B	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-C	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-D	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-E	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-F	L W D Q S L K P C V K L T	<u>SEQ ID NO:4</u>
Consensus-G	L W D E S L K P C V K L T	<u>SEQ ID NO:5</u>
Consensus-O	L W D Q S L K P C V Q M T	<u>SEQ ID NO:6</u>
Consensus-U	L W D ? S L K P C V K L T	<u>SEQ ID NO:7</u>
Peptide 113	S V I T Q A C S K V S F E	<u>SEQ ID NO:8</u>
Consensus-A	S A I T Q A C S K V S F E	<u>SEQ ID NO:9</u>
Consensus-B	S V I T Q A C S K V S F E	<u>SEQ ID NO:8</u>
Consensus-C	S A I T Q A C S K V S F D	<u>SEQ ID NO:10</u>
Consensus-D	S A I T Q A C S K V T F E	<u>SEQ ID NO:38</u>
Consensus-E	S V I K Q A C S K I S F D	<u>SEQ ID NO:11</u>
Consensus-F	S T I T Q A C S K V S W D	<u>SEQ ID NO:12</u>
Consensus-G	S T I K Q A C S K V N F D	<u>SEQ ID NO:13</u>
Consensus-O	T T I ? Q A C S K V S F E	<u>SEQ ID NO:14</u>
Consensus-U	S ? I K Q A C S K V S F E	<u>SEQ ID NO:15</u>
Peptide 116	G T G P C T N V S T V Q C	<u>SEQ ID NO:16</u>
Consensus-A	G T G P C K N V S T V Q C	<u>SEQ ID NO:17</u>
Consensus-B	G T G P C T N V S T V Q C	<u>SEQ ID NO:16</u>
Consensus-C	G T G P C H N V S T V Q C	<u>SEQ ID NO:18</u>
Consensus-D	G T G P C K N V S T V Q C	<u>SEQ ID NO:17</u>
Consensus-E	G T G P C K N V S S V Q C	<u>SEQ ID NO:39</u>
Consensus-F	G T G P C K N V S T V Q C	<u>SEQ ID NO:17</u>
Consensus-G	G T G P C K N V S T V Q C	<u>SEQ ID NO:17</u>
Consensus-O	G T G L C ? N I T V V T C	<u>SEQ ID NO:19</u>
Consensus-U	G T G P C K N V S T V Q C	<u>SEQ ID NO:17</u>
Peptide 63	F L G F L G A A G S T M G A A S L T L T V Q A R Q	<u>SEQ ID NO:20</u>
Consensus-A	F L G F L G A A G S T M G A A S I T L T V Q A R Q	<u>SEQ ID NO:21</u>
Consensus-B	F L G F L G A A G S T M G A A S ? T L T V Q A R Q	<u>SEQ ID NO:22</u>
Consensus-C	F L G F L G A A G S T M G A A S L T L T V Q A R Q	<u>SEQ ID NO:20</u>

Consensus-D	FLGFLGAAGSTMGAAS?TLTVQARQ	<u>SEQ ID NO:22</u>
Consensus-E	IFGFLGAAGSTMGAASLTLLTVQARQ	<u>SEQ ID NO:23</u>
Consensus-F	FLGFLGAAGSTMGAASLTLLTVQARQ	<u>SEQ ID NO:20</u>
Consensus-G	FLGFLGAAGSTMGAAAATLTVQARQ	<u>SEQ ID NO:24</u>
Consensus-O	FLGVLSAAGSTMGAASLTLLTVQARQ	<u>SEQ ID NO:40</u>
Consensus-U	FLGFLGAAGSTMGAAS??LTQARQ	<u>SEQ ID NO:25</u>

Peptide 61	YLRDQQQLLGIGWG	<u>SEQ ID NO:26</u>
Consensus-A	YLRDQQQLLGIGWG	<u>SEQ ID NO:26</u>
Consensus-B	YLKDQQQLLGIGWG	<u>SEQ ID NO:27</u>
Consensus-C	YLKDQQQLLGIGWG	<u>SEQ ID NO:27</u>
Consensus-D	YLKDQQQLLGIGWG	<u>SEQ ID NO:27</u>
Consensus-E	YLKDQKFLGLWG	<u>SEQ ID NO:28</u>
Consensus-F	YL?DQQQLLGIGWG	<u>SEQ ID NO:29</u>
Consensus-G	YL?DQQQLLGIGWG	<u>SEQ ID NO:30</u>
Consensus-O	YLRDQQQLLGIGWG	<u>SEQ ID NO:31</u>
Consensus-U	YLESQQLLGIGWG	<u>SEQ ID NO:32</u>

Please substitute Table 4 on page 85 for:

TABLE 4. Peptide-Specificity of CTL Response in an HIV-Seropositive LTNP

Peptide No.	Residues	Peptide amino acid sequence ²	100:1	50:1	25:1	12.5:1	% Specific lysis at various E:T ratios ³
61	586-598	<u>YLRDQQLLGIWGC (SEQ ID NO:33)</u>	15.0	14.5	4.0	4.0	
63	519-543	<u>FLG[H]FLGAAGSTMGAASLTL TVQARQ (SEQ ID NO:20)</u>	17.6	8.8	7.9	0	
104	44-55	<u>VYYGVPVWKAE (SEQ ID NO:1)</u>	12.2	9.3	3.7	1.5	
113	204-216	<u>SVITQACSKVVSFE (SEQ ID NO:8)</u>	0	0	0	0	
		Medium					
		vSC-8 ⁴	2.9	2.2	3.0	1.6	
		vPE-16 ⁵	22.4	19.5	16.4	3.5	

¹The CTL activity was assayed using PBMCs from patient volunteer HD.

²The amino acid sequence of peptides was according to Modrow *et al.*, *J. Virol.*, 61:570-578, 1987.

³CTL activity of PBMCs restimulated for 7 days by growth in phytohemagglutinin medium was assayed against peptide-pulsed autologous EBV-transformed B cells (B-LCL).

⁴Freshly isolated PBMCs were assayed for CTL activity against B-LCL infected with control vaccinia virus.

⁵Freshly isolated PBMCs were assayed for CTL activity against B-LCL infected with recombinant vaccinia virus expressing gp160 from HIV-1 IIIB.

Please substitute Table 5 on page 87 for:

TABLE 5
**Amino acid sequence of synthetic peptides from conserved regions
in the HIV-1 envelope protein**

Peptide no.	Amino acid residues	Amino acid sequence*
104	45 - 55	VYYGVPVWKEA (<u>SEQ ID NO:1</u>)
113	204 - 216	SVITQACSKVSFE (<u>SEQ ID NO:8</u>)
120	586 - 598	YLRDQQQLGIWG (<u>SEQ ID NO:33</u>)
121	519 - 543	FLGFLGAAGSTMGAASLTQTVQARQ (<u>SEQ ID NO:20</u>)
122	417 - 435	CRIKQIINMWQGVGKAMYA (<u>SEQ ID NO:36</u>)

* The amino acid sequence of peptides was according to Modrow *et al.*

Please substitute the paragraph extending from page 87 line 22 through page 89 line 7 for:

These peptides corresponding to conserved regions in the envelope protein of HIV-1 were identified as T-cell epitopes in mice and rhesus monkeys in our previous studies (Nehete, 1993; Sastry, 1991). Peptides were synthesized as described before (Sastry, 1991) using the Merrifield solid-phase method (Merrifield, 1963) either on a modified Vega 250 automatic peptide synthesizer (Vega Biochemical, Tucson, AZ) or by the "bag" method as described by Houghten (Houghten, 1985). In most of the experiments, the purity of the peptides used was approximately 70-80%, and in limited experiments, peptides exhibiting >95% purity were used with identical results. In addition to the conserved envelope peptides, a peptide from the c-mos proto-oncogene (aa 158-170, STRTPEDSNSLGT (SEQ ID NO:37)) was used as a control in all the experiments. Additional control peptides used in majority of the experiments included: a peptide from the c-abl protooncogene, peptides from E6 and E7 oncoproteins of HPV-16, a peptide corresponding to the V3-loop region in gp120 but with a scrambled amino acid sequence, and peptides from the pol and gag regions of HIV-1. In all these cases, the amount of proliferative responses to the controls was consistently and significantly less than the amount of

response the test peptides from the HIV envelope protein. In all these cases, the level of proliferative responses [tot he] to the control peptide was consistently and significantly less than the level of response [to the] to the HIV envelope protein. The c-mos peptide was consistently used in all the experiments as a control. Stock solutions of peptides were prepared in phosphate buffered saline (PBS) (pH 7.0) and filter sterilized.

Please substitute Table 8 on page 96 for:

TABLE 8
Conserved HIV envelope peptides used in the vaccine study

Peptide no.	Amino acid residues	Amino acid sequence
61	586 - 597	YLRDQQLLGIWG <u>(SEQ ID NO:33)</u>
63	519 - 543	FLGFLGAAGSTMGAASLTLTVQARQ <u>(SEQ ID NO:20)</u>
104	45 - 55	VYYGVPVWKEA <u>(SEQ ID NO:1)</u>
111	118 - 130	LWDQSLKPCVKLT <u>(SEQ ID NO: 4)</u>
113	204 - 216	SVITQACSKVSFE <u>(SEQ ID NO:8)</u>
116	540 - 552	GTGPCTNVSTVQC <u>(SEQ ID NO:16)</u>